DETERMINATION OF FREE AND GLYCOSIDICALLY BOUND MALEIC HYDRAZIDE IN TOBACCO

DETERMINATION DE L'HYDRAZIDE MALEIQUE LIBRE ET SOUS FORME DE GLYCOSIDE DANS LE TABAC

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SUMMARY

Maleic hydrazide (MH), a systemic plant-growth regulator is used extensively for tobacco-sucker control. Due to the high residue levels found on raw-leaf tobacco, the development of a fast, specific and simple procedure is of interest for the determination of MH on tobacco. A new method was established involving the methylation of MH with dimethyl sulfate and the gas chromatographic quantitation of the derivative using a specific nitrogen-phosphorous detector. Results obtained by the GC procedure are in accordance with the values obtained by the colorimetric (ISO) procedure.

RESUME

L'hydrazide maléique (HM) est un régulateur systèmique de croissance qui est utilisé extensivement pour le traitement inhibiteur des bourgeons du tabac. Etant donné les valeurs élevées des résidus détectés sur les feuilles de tabac, le dévelopement d'une méthode rapide, spécifique et simple rencontre un grand intérêt. Une nouvelle procédure a été mise au point qui inclut la méthylation de HM avec le diméthyl sulfate, suivie de la quantification du dérivé par chromatographie gazeuse en utilisant un détecteur spécifique azote-phosphore. Les résultats

obtenus selon la nouvelle procédure sont en accord avec les résultats trouvés par la méthode colorimétrique (ISO).

INTRODUCTION

Maleic hydrazide (3.6-dioxo-1.2-dihydro pyridazine) (MH) is a systemic plant growth-regulator which is used extensively for tobacco-sucker control. MH is usually applied to the upper half or third of the tobacco plant within 24 h after topping. It is subsequently found throughout the plant due to absorption and translocation. MH inhibits cell division without affecting cell elongation, thus preventing the growth of newly developing suckers without retarding the growth of more mature leaves. A β-D-glucoside, representing 15 % of the free MH fraction, was identified in tobacco, wheat and apple [1]. Results from relevant literature [2] show that average MH residues on US flue-cured tobaccos have remained constantly above 100 ppm since about 1974, and individual values on raw-leaf tobacco up to 300 ppm were measured. Average values obtained in our laboratories on the latest crops, i.e., 1985 to 1989, confirm the published values. As a consequence, residues found on American cigarettes are significant. For example, an increase from 39 ppm in 1973 to 66 ppm in 1984 was observed [2].
Despite the fact that no evidence of harmful effects was obtained [2], a maximum amount of 80 ppm has been recommended in Germany which requires an analytical procedure for MH determination on tobacco.
This determination is now being performed by a colorimetric procedure which was adopted as an ISO Standard Method [3] and an official method of the Association of Official Analytical Chemists [4]. The procedure consists in a hydrolytic reduction of MH to hydrazine by zinc in an aqueous sodium hydroxide solution. The hydrazine is subsequently steam distilled and collected in an acidic solution of p-dimethyl-aminobenzaldehyde to form a yellow compound with an absorption maximum at 455 nm.

For the determination of total MH, i.e., free and glucosidic, an acidic digestion precedes the reduction with Zn. The colorimetric method has several drawbacks : interferences from several leaf constituents are possible [5]; the procedure is time-consuming and relatively difficult to handle. Therefore, a specific and more simple procedure is needed for MH determination on tobacco. This paper describes a method using acidic hydrolysis, methylation and gas chromatography with a Nitrogen-Phosphorous detector for the quantitative and unambiguous determination of MH on tobacco.

EXPERIMENTAL SECTION

Apparatus

Gas chromatograph, Carlo Erba 5160 equipped with Nitrogen-Phosphorous detector (NPD) and connected to a Spectra-Physics DP-700 integrator.

GC column, 30 m x 0.25 mm i.d. fused silica column, coated with 0.25 um of DB-17, J&W.

Gas chromatograph - mass spectrometer, Hewlett Packard 5988 mass spectrometer, interfaced with a Hewlett Packard 59970 data system and a Hewlett Packard 5890 gas chromatograph.

NMR spectrometer, Bruker WP-200

Reagents

- Maleic hydrazide (MH) 99 %, Riedel-de-Haën
- 5.5-dimethylbarbituric acid (barbital), puriss p.a., Fluka
- Hydrochloric acid, puriss p.a., 32 %, Fluka
- Dimethyl sulfate, puriss p.a., Fluka
- Potassium carbonate, p.a., Merck
- Sodium hydroxyde, puriss p.a., Fluka
- Chloroform, puriss p.a., Fluka
- Extracting solution : barbital in 2N hydrochloric acid, 1 mg/l
- Calibration solution : maleic hydrazide in extracting solution, 1 mg/l.

Sample preparation

Filler and tobacco leaves : 5 g of filler or ground tobacco leaves are introduced into a 250-ml flask and extracted with 100 ml of extracting solution for 2 h under reflux. After cooling, the extract is filtered through a folded filter, a 1-ml aliquot is taken for derivatization and introduced into a 5-ml reaction vessel.

Standard : 1 ml of calibration solution is introduced into a 5-ml reaction vessel and processed in parallel with the tobacco extracts.

Standard for GC-MS and 1H-NMR analyses : 1 ml of a 10-mg/ml solution of maleic hydrazide in 2 N HCl is introduced into a 5-ml reaction vessel. Derivatization is performed with 1 ml of dimethyl sulfate according to the procedure described below.

Derivatization and extraction

After neutralizing the extracting solution with NaOH 10 N and bringing its pH up to 11 with K2CO3 sat., 0.2 ml of dimethyl sulfate are added. The solution is shaken vigorously and let react at 75°C for 1 h. After cooling, 500 ul of chloroform are added, the flask is shaken vigorously by hand for 3 min and finally centrifuged (1 min at 3000 rpm). The organic layer is introduced into a 200-ul GC vial. 1 ul of the chloroform solution is injected into the GC.

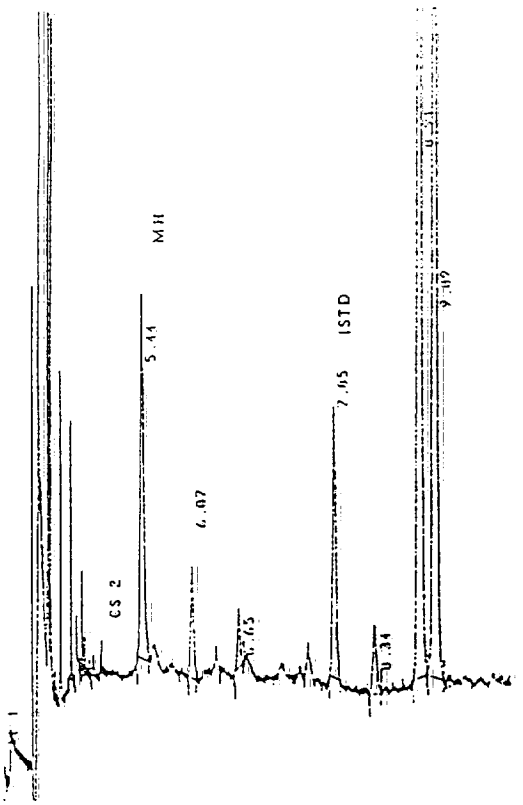
Gas chromatography

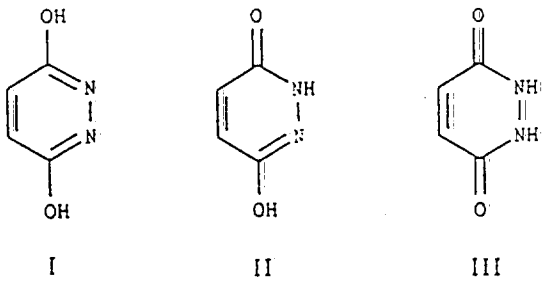
GC conditions were as follows : split injector (1:30), 250°C; NP detector, 270°C; temperature program : 140°C, 3 min/ 5°C/min / 200°C / 20°C/min / 250°C, 16 min.

3 calibration replicates are first injected. The quantitation of samples is then performed using the internal standard method. MH residues are expressed as mg/g (dry weight tobacco basis).

RESULTS AND DISCUSSION

Due to its polarity, maleic hydrazide cannot be extracted from the aqueous hydrolysis solution with an organic solvent. The derivative obtained by reaction with dimethyl sulfate is highly soluble in $CHCl_3$ and therefore can be easily extracted and analysed by gas chromatography (Fig. 1).





I II III

Maleic hydrazide could exist in any of the 3 tautomeric forms I-III (Fig. 2).

Katritzky and al [6] demonstrated that II is clearly predominent. This structure shows the presence of one hydroxy and one secondary amine group which are suitable for methylation. The methylation of MH with dimethyl sulfate results in the quantitative formation of the N-O-dimethyl derivative corresponding to isomer II. The positive proof of the identity of the derivative was obtained by GC-MS and 1H-NMR. In the mass spectrum, the molecular ion at m/z 140 corresponding to the N,O-dimethyl

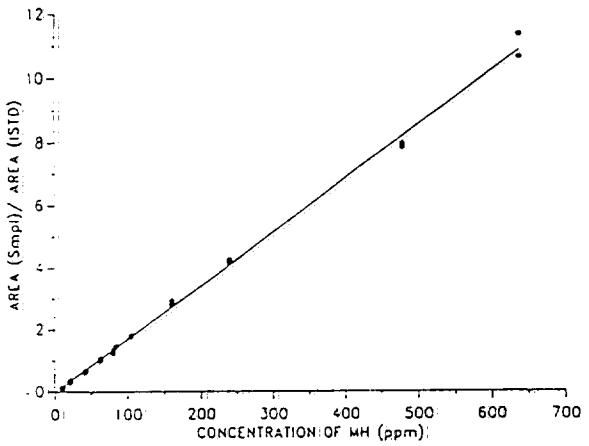
derivative is very abundant (92 %) and its fragmentation gives main signals at m/z 112 (36%), 80 (47%) and 69 (100%) which is the base peak of the spectrum. The molecular ion loses a molecule of CO producing an ion at m/z 112 which loses formaldehyde, resulting in a peak at m/z 80. The base peak at m/z 69 results from the successive losses of CH_3, N_2 and CO from the molecular ion.

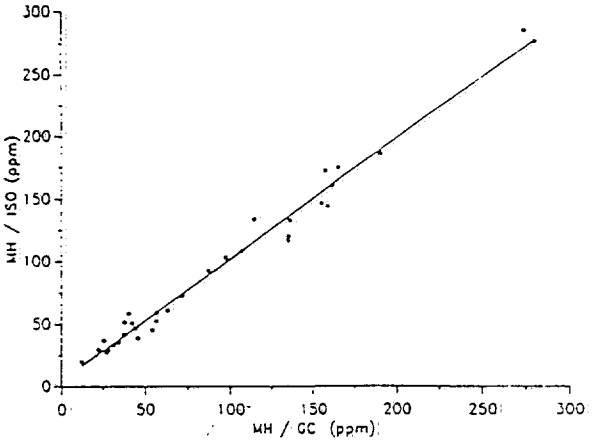
The 1H-NMR spectrum consists in the following 3 signals : δ =3.6 ppm, S, 3H, CH_3-N; δ= 3.8 ppm, S, 3H, CH_3-O; δ =6.9 ppm, S, 2H, H-C=C-H. The presence of 2 distinct signals at 3.6 and 3.8 ppm corroborates the information from the mass spectrum showing that the derivative obtained corresponds to tautomer II.

Barbital was chosen as internal standard because of the similarity of its structure with that of MH. This ensures that both compounds react and can be extracted similarly. The reaction of barbital with dimethyl sulfate produces the N,N-dimethyl derivative. This reaction was described by Martin [7] in 1966.

The NP-detector response is linear (r^2 = 0,999) within the whole range tested, i.e., from 10 to 600 ppm as shown in Fig.3.

Recoveries of MH from tobacco never treated with MH in the field when fortified with 80 ppm average 95 %. A relative standard deviation of 7 % was obtained at both 10 and 70 ppm on tobacco samples. The detection limit of the method determined on cigarette filler is 5 ppm. A comparative study between results obtained by the GC and colorimetric (ISO) procedure was performed. 65 samples including commercial cigarettes as well as tobacco leaves were analysed by both methods : ISO values reported as a function of the GC values (Fig.4) show a good agreement between both procedures. The slope of the correlation line is 0.972, the intercept +4.5 ppm and the correlation coefficient r^2 = 0.985. The 4.5 ppm intercept may result from the lower specificity of the ISO method.





The bottleneck of the method is the hydrolysis step which is time-consuming and requires a lot of glassware. At present, the number of samples which can be processed everyday by one person is limited to 20. However, the daily capacity of the GC method is at least twice as high as the colorimetric one and could be increased greatly if, for example, the acid hydrolysis could be replaced by an enzymatic one.

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